Electronic Supplementary Material (ESI) for Environmental Science: Nano.

-Electronic Supplementary Information-

Important ecological processes are affected by the accumulation and trophic transfer of nanoplastics in a freshwater periphyton-grazer food chain

Manuel Holzer[a], Denise M. Mitrano[b], Louis Carles[a], Bettina Wagner[a] and Ahmed Tlili[a,*]

[a] *Department of Environmental Toxicology, Swiss Federal Institute of Aquatic Science and Technology (Eawag), 8600 Dübendorf, Switzerland*

[b] *Department of Environmental Systems Science, ETH Zurich, Universitatstrasse 16, 8092 Zurich, Switzerland*

*Corresponding author: Ahmed Tlili

Address: Eawag, Department of Environmental Toxicology

Überlandstrasse 133. 8600 Dübendorf, Switzerland 14

Phone: + 41 58 765 5330 15

Email: ahmed.tlili@eawag.ch

Table S1. Composition of the exposure medium PERIQUIL

Component	**Concentration (mM)**
Salts	
$CaCl_2$	0.20
Ca(NO3)2	0.10
$MgSO_4$	0.15
$NaHCO_3$	1.20
KNO_3	0.10
Na_2SiO_3	0.05
Nutrients	
K_2HPO_4	5.00×10^{-3}
NH_4NO_3	0.10
Trace elements	
$CoCl_2$	5.00×10^{-5}
H_3BO_3	0.05
Na_2MoO_4	8.00×10^{-5}
$CuSO_4$	1.63×10^{-4}
$MnCl_2$	1.22×10^{-3}
$ZnSO_4$	1.58×10^{-4}
$FeCl_3$	9×10^{-4}
Metal ligand	
Na_2 EDTA	0.02
Buffer	
MOPS (pH 7.5)	10.00
NaOH	7.00

Table S2. Read processing reports for 16S and 18S rRNA genes. Read counts for each sample for each of the processing steps. The mean length of the reads is also indicated in the table. The composition of Mock communities used as positive controls is described in Carles et al. (https://doi.org/10.1016/j.watres.2021.117486).

						Read counts				
Target	Sample	Sample material	Treatment	Time	Replicate	Raw	Merged	Primer	Clean	Mean read length (bp)
16S rRNA gene	B21-16S	Biofilm	Control	Day 0	R01	225658	222166	216880	211062	413.849
	B22-16S	Biofilm	Control	Day 0	R02	240121	236142	230288	224137	413.807
	B23-16S	Biofilm	Control	Day 0	R03	219808	216539	210846	205365	413.842
	B24-16S	Biofilm	Control	Day 0	R04	231122	227285	220627	214576	413.169
	B25-16S	Biofilm	Control	Day 0	R05	205869	202911	197496	192203	415.139
	B26-16S	Biofilm	nanoPS	Day 0	R01	153177	150632	147040	143214	415.414
	B27-16S	Biofilm	nanoPS	Day 0	R02	140017	136821	132426	128963	416.371
	B28-16S	Biofilm	nanoPS	Day 0	R03	156248	153805	150041	145992	414.174
	B29-16S	Biofilm	nanoPS	Day 0	R04	98381	91655	86710	84564	414.572
	B30-16S	Biofilm	nanoPS	Day 0	R05	199257	196127	192068	187002	416.685
	B31-16S	Biofilm	Control	Day 15	R01	159237	156891	152581	148458	415.864
	B32-16S	Biofilm	Control	Day 15	R02	180529	177721	173447	168763	416.313
	B33-16S	Biofilm	Control	Day 15	R03	163206	160610	155705	151471	416.341
	B34-16S	Biofilm	Control	Day 15	R04	167899	165093	161379	157068	416.91
	B35-16S	Biofilm	Control	Day 15	R05	202244	198854	193884	188697	416.14
	B36-16S	Biofilm	nanoplastics	Day 15	R01	207772	204533	199432	194147	416.517
	B37-16S	Biofilm	nanoplastics	Day 15	R02	213434	209799	203939	198248	416.437
	B38-16S	Biofilm	nanoplastics	Day 15	R03	221851	218121	213030	207392	416.882
	B39-16S	Biofilm	nanoplastics	Day 15	R04	165251	162473	158476	154165	416.743
	B40-16S	Biofilm	nanoplastics	Day 15	R05	173850	170720	166094	161595	416.55
	B41-16S	Biofilm	Snail	Day 15	R01	140206	137762	134390	130768	421.822
	B42-16S	Biofilm	Snail	Day 15	R02	162296	159386	155111	150978	422.368
	B43-16S	Biofilm	Snail	Day 15	R03	163832	161091	157025	152942	419.561
	B44-16S	Biofilm	Snail	Day 15	R04	172928	169981	165681	161314	420.95
	B45-16S	Biofilm	Snail	Day 15	R05	182476	179671	175285	170511	420.472

	B46-16S	Biofilm	nanoPS×Snail	Day 15	R01	146893	144061	140456	136671	418.881
	B47-16S	Biofilm	nanoPS×Snail	Day 15	R02	149829	147232	143346	139475	417.826
	B48-16S	Biofilm	nanoPS×Snail	Day 15	R03	190937	187392	182520	177556	418.921
	B49-16S	Biofilm	nanoPS×Snail	Day 15	R04	176864	173941	169220	164655	419.848
	B50-16S	Biofilm	nanoPS×Snail	Day 15	R05	150751	147830	143694	139795	418.211
									Total = 4991747	
	NegExtr1-16S	Negative control	-	-	-	676	412	382		424.127
	PosPCR-16S	Positive control	-	-	-	172064	169651	166313		426.153
18S rRNA gene	B21-18S	Biofilm	Control	Day 0	R01	193140	188008	187965	187616	274.999
	B22-18S	Biofilm	Control	Day 0	R02	169023	164367	164172	163730	274.998
	B23-18S	Biofilm	Control	Day 0	R03	153421	149299	149232	148838	274.998
	B24-18S	Biofilm	Control	Day 0	R04	99036	95222	95151	94786	274.997
	B25-18S	Biofilm	Control	Day 0	R05	168849	164865	164654	164271	274.998
	B26-18S	Biofilm	nanoplastics	Day 0	R01	154303	150502	150351	149924	274.998
	B27-18S	Biofilm	nanoplastics	Day 0	R02	182252	177662	177602	177287	274.998
	B28-18S	Biofilm	nanoplastics	Day 0	R03	163990	159945	159842	159537	274.998
	B29-18S	Biofilm	nanoplastics	Day 0	R04	160325	156218	156120	155881	274.998
	B30-18S	Biofilm	nanoplastics	Day 0	R05	176120	171296	171217	170774	274.998
	B31-18S	Biofilm	Control	Day 15	R01	127360	123917	123888	123678	274.998
	B32-18S	Biofilm	Control	Day 15	R02	145988	142146	142028	141337	274.998
	B33-18S	Biofilm	Control	Day 15	R03	33051	31297	29980	29907	274.991
	B34-18S	Biofilm	Control	Day 15	R04	23652	22296	21527	21476	274.987
	B35-18S	Biofilm	Control	Day 15	R05	43081	40320	39033	38988	274.993
	B36-18S	Biofilm	nanoplastics	Day 15	R01	57168	54220	53279	53227	274.995
	B37-18S	Biofilm	nanoplastics	Day 15	R02	60794	57403	56512	56407	274.995
	B38-18S	Biofilm	nanoplastics	Day 15	R03	55003	52024	51246	51168	274.995
	B39-18S	Biofilm	nanoplastics	Day 15	R04	98554	92626	90718	90598	274.997
	B40-18S	Biofilm	nanoplastics	Day 15	R05	80188	75733	74054	73899	274.996
	B41-18S	Biofilm	Snail	Day 15	R01	5034	4163	3277	3270	274.916
	B42-18S	Biofilm	Snail	Day 15	R02	5012	4324	3626	3608	274.924
	B43-18S	Biofilm	Snail	Day 15	R03	21836	20358	19579	19512	274.986
	B44-18S	Biofilm	Snail	Day 15	R04	10269	9501	8631	8619	274.968

B45-18S	Biofilm	Snail	Day 15	R05	38270	36072	35635	35563	274.992
B46-18S	Biofilm	nanoPS×Snail	Day 15	R01	17913	16656	15704	15673	274.982
B47-18S	Biofilm	nanoPS×Snail	Day 15	R02	82992	79019	77282	77142	274.996
B48-18S	Biofilm	nanoPS×Snail	Day 15	R03	46135	42008	40386	40313	274.993
B49-18S	Biofilm	nanoPS×Snail	Day 15	R04	39904	37229	35516	35441	274.992
B50-18S	Biofilm	nanoPS×Snail	Day 15	R05	38808	35678	34702	34589	274.992
								Total = 2527059	
NegExtr2-18S	Negative control	-	-	-	1687	1628	77	69	271.071
PosPCR-18S	Positive control	-	-	-	94705	92083	90376	90323	274.997

Table S3. Two-way ANOVA results of periphyton endpoints at the end of the feeding trial at day 14.

	Snail effect	nanoplastics effect	nanoplastics × Snail effect
AFDW	ns	ns	ns
Chlorophyll-a	$F = 9.5$, $P < 0.007$	$F = 36.8$, $P < 0.0001$	ns
Photosynthetic efficiency	$F = 53.4$, $P < 0.0001$	$F = 14.1$, $P < 0.002$	ns
Lipid content	ns	ns	ns
Carbon:Nitrogen	ns	ns	ns
Carbon:Phosphorus	ns	ns	ns
Nitrogen:Phosphorus	ns	ns	ns

Table S4. Measured endpoints (mean ± SD, n = 5) at day 0 and day 14 (i.e., start and end of the feeding trial) of periphyton (previously exposed for 24 hours to medium without (control) or with the filtrate of 0.5 mg nanoparticles L^{-1} suspension (f-nanoplastics)) in the presence or absence of the grazers (snail).

	Day 0		Day 15			
	Control	f-nanoplastics	Control	Snail	f-nanoplastics	f-nanoplastics × Snail
Biomass						
AFDW (g m^2)	4.0 ± 0.8	5.1 ± 1.0	4.6 ± 1.3	1.9 ± 0.1	4.3 ± 1.1	2.5 ± 0.8
chlorophyll-a (mg g^{-1} AFDW)	17.2 ± 2.4	16.3 ± 2.5	2.6 ± 0.7	0.8 ± 0.2	1.9 ± 0.6	5.5 ± 1.3
Physiology						
photosynthetic efficiency (quantum yield ϕ')	0.55 ± 0.06	0.58 ± 0.08	0.57 ± 0.04	0.56 ± 0.03	0.55 ± 0.04	0.58 ± 0.04
Nutrient value						
lipid content (mg glycerol g^{-1} AFDW)	308 ± 99	195 ± 38	266 ± 107	357 ± 121	258 ± 78	468 ± 196

Table S5. Two-way ANOVA results of periphyton endpoints from the control experiment with the filtrate of the 0.5 mg L^{-1} nanoplastics suspension

	Snail effect	f-nanoplastics effect	f-nanoplastics × Snail effect
AFDW	F = 28.5, P < 0.0001	ns	ns
Chlorophyll-a	F = 9.4, P = 0.007	ns	ns
Photosynthetic efficiency	ns	ns	ns
Lipid content	F = 6.4, P = 0.022	ns	ns

Table S6. Two-way ANOVA and pairwise comparisons results of periphyton alpha-diversity at day 14, corresponding to the end of the feeding trial.

Two-way ANOVA		nanoplastics effect	Snail effect	nanoplastics × Snail effect
Prokaryotes	Chao 1	F = 11.0, P = 0.0044	F = 42.32, P < 0.0001	F = 8.244, P = 0.0111
	Shannon index	F = 9.4, P = 0.0072	F = 49.76, P < 0.0001	F = 9.888, P = 0.0063
Eukaryotes	Chao 1	ns	F = 40.11, P < 0.0001	F = 8.168, P = 0.0114
	Shannon index	ns	F = 11.49, P = 0.0037	ns

Pairwise comparisons		Result	
		Chao 1	Shannon index
Prokaryotes	Control *vs* . nanoplastics	ns	ns
	Control *vs* . Snail	F = 9.3, P < 0.0001	F = 10.2, P < 0.0001
	Control vs. nanoplastics×Snail	ns	ns
	nanoplastics vs. Snail	F = 9.8, P < 0.0001	F = 10.1, P < 0.0001
	nanoplastics vs. nanoplastics×Snail	ns	ns
	Snail vs. nanoplastics×Snail	F = 6.2, P = 0.0024	F = 6.2, P = 0.0023
Eukaryotes	Control *vs* . nanoplastics	ns	ns
	Control *vs* . Snail	F = 9.2, P < 0.0001	ns
	Control vs. nanoplastics×Snail	F = 5.8, P = 0.0041	F = 5.1, P = 0.012
	nanoplastics vs. Snail	F = 6.8, P = 0.0009	ns
	nanoplastics vs. nanoplastics×Snail	ns	ns
	Snail vs. nanoplastics×Snail	ns	ns

Table S7. Two-way PERMANOVA and pairwise comparisons results of periphyton β-diversity at day 14, corresponding to the end of the feeding trial. Weighted UniFrac distances were used to evaluate β-diversity.

Two-way PERMANOVA		nanoplastics effect	Snail effect	nanoplastics × Snail effect
Prokaryotes		ns	$F = 23.4$, $P < 0.01$	$F = 4.4$, $P < 0.05$
Eukaryotes		ns	$F = 11.3$, $P < 0.01$	$F = 2.6$, $P < 0.05$
Pairwise comparisons		**Result**		
Prokaryotes	Control *vs* . nanoplastics	ns		
	Control *vs* . Snail	$F = 14.9$, $P < 0.05$		
	Control vs. nanoplastics×Snail	$F = 6.7$, $P < 0.05$		
	nanoplastics vs. Snail	$F = 21.4$, $P < 0.05$		
	nanoplastics vs. nanoplastics×Snail	$F = 12.2$, $P < 0.05$		
	Snail vs. nanoplastics×Snail	$F = 4.1$, $P < 0.05$		
Eukaryotes	Control *vs* . nanoplastics	ns		
	Control *vs* . Snail	$F = 5.1$, $P < 0.05$		
	Control vs. nanoplastics×Snail	$F = 8.6$, $P < 0.05$		
	nanoplastics vs. Snail	$F = 5.4$, $P < 0.05$		
	nanoplastics vs. nanoplastics×Snail	$F = 9.8$, $P < 0.05$		
	Snail vs. nanoplastics×Snail	$F = 2.8$, $P < 0.05$		

Table S8. Two-way ANOVA results of the abundance of the top-ten prokaryotic and eukaryotic phyla in periphyton at day 14, corresponding to the end of the feeding trial.

		nanoplastics effect	Snail effect	nanoplastics × Snail effect
Prokaryotes	Acidobacteria	ns	$F = 16.2$, $P < 0.001$	$F = 5.2$, $P < 0.05$
	Armatimonadetes	ns	$F = 25.6$, $P < 0.001$	ns
	Bacteroidetes	ns	ns	ns
	Cyanobacteria	ns	ns	ns
	Deinococcus-Thermus	ns	ns	ns
	Gemmatimonadetes	ns	$F = 77.4$, $P < 0.001$	ns
	Parcubacteria	$F = 11.8$, $P < 0.01$	$F = 96.5$, $P < 0.001$	ns
	Planctomycetes	ns	ns	ns
	Proteobacteria	ns	$F = 5.2$, $P < 0.05$	$F = 11.4$, $P < 0.01$
	Verrucomicrobia	ns	ns	$F = 9.6$, $P < 0.01$
Eukaryotes	Aphelida	ns	$F = 5.9$, $P < 0.05$	ns
	Bacillariophyta	ns	$F = 19.4$, $P < 0.001$	$F = 5.2$, $P < 0.05$
	Cercozoa	ns	$F = 9.9$, $P < 0.01$	ns
	Chlorophyta	ns	ns	$F = 4.8$, $P < 0.05$
	Chytridiomycota	ns	ns	ns
	Ciliophora	$F = 6.6$, $P < 0.05$	$F = 30.6$, $P < 0.001$	ns
	Discosea	$F = 7.7$, $P < 0.05$	$F = 9.1$, $P < 0.01$	ns
	Nematoda	ns	ns	ns
	Rotifera	ns	$F = 8.5$, $P < 0.05$	ns
	Stramenopiles	ns	$F = 26.4$, $P < 0.001$	ns

Table S9. Reproductive output (mean ± SD) of snails during 14 days of feeding on periphyton previously exposed for 24 hours to medium without (Control) or with the filtrate (f-nanoplastics) of 5 mg nanoplastics L^{-1} suspension. Means and SD are calculated from five replicate microcosms per treatment, each containing four snails.

Treatment	Number of egg clutches	Number of eggs	Number of eggs per clutch
Control	17 ± 6 (87)	220 ± 74 (1101)	13 ± 4
f-nanoplastics	11 ± 5 (55)	150 ± 64 (748)	14 ± 4